A Simple Quantitative Procedure for Obtaining the Unsaponifiable Matter from Butteroil

D. P. SCHWARTZ, L. H. BURGWALD and C. R. BREWINGTON, Dairy Products Laboratory, Eastern Utilization Research and Development Division, ARS, USDA, Washington, D.C.

Abstract

A simple, relatively rapid quantitative procedure for obtaining the unsaponifiable matter from butteroil is described. The oil is saponified without a solvent by merely blending the oil with aqueous KOH in a mortar and heating. The unsaponifiable matter is extracted from the soaps with benzene after grinding the soaps onto Celite.

DURING AN INVESTIGATION of the hydroxyl components in various fractions of butteroil, a saponification procedure was needed which obviated the use of an alcoholic medium. Although a number of variations of the Official Amercian and British methods have been proposed, (1,2) no reference to the use of a nonalcoholic solvent in the actual saponification step could be found. We, therefore, attempted to develop a saponification procedure in which no solvent was employed; first, by carrying out the saponification on the surface of an inert support with some success, and later, by simply blending the oil with the alkali. The latter procedure repeatedly gave complete saponification, and the matter of quantitative isolation of the unsaponifiable matter from the soap was subsequently perfected.

Experimental

Preparation of Butteroil

Freshly pasteurized cream (obtained from mixed herd milk, Beltsville, Maryland) was churned in an electric drink mixer, the butter isolated, melted at 52C and stored overnight at 4C. The butter, was remelted at 63C and the buttermilk removed. The fat phase was centrifuged at 63C and the buttermilk removed. The fat phase was centrifuged at 5000 rpm (International Centrifuge, Size 1, Type SB) and the clear oil pipetted off.

Quantitative technique is used throughout the rest of the procedure.

Saponification

Potassium hyroxide pellets (3 g) and 1.5 ml. of distilled water are ground together with a pestle in a 4 inch mortar. Between 4 and 5 g of butteroil is transferred from a weighed vessel to the mortar and the exact weight of the oil taken is determined. The oil is thoroughly ground with the alkali until soap formation visibly commences (about 5 min) and saponification is completed by placing the mortar containing the pestle in a 100C oven for 20 min.

Extraction

At the end of the saponification period the mortar is removed from the oven, covered with aluminum foil, and cooled sufficiently to be handled comfortably. To facilitate extraction of the unsaponifiables from the soap, the latter is ground in the mortar with 9 g of dried (100C for at least 24 hr) Celite 545 and 1 g of powdered anhydrous $CaCl_2$. It is important that all of the soap be adsorbed by the Celite and that the resultant powder be homogeneous. This is best accomplished by grinding, scraping the pestle and mortar with a flat spatula and a spoon, regrinding, rescraping and repeating these operations until the powder appears homogeneous. This procedure takes about 15 min and with a little experience homogeneity and subsequent quantitative recovery of the unsaponifiables is attained. The powder is transferred to a coarse sintered glass funnel (4 cm I.D., 60 ml. capacity) and packed by tapping the stem several times on the bench top. The mortar, pestle, spoon, and spatula are rinsed with 10 ml of benzene (Fisher, crystallizable grade) the rinsings transferred to the funnel and permitted to completely drain into the column. Thereafter, two 40-ml portions of benzene are added. This volume of benzene is usually sufficient to extract all of the unsaponifiables, but as a check another 5 ml of benzene is added, the effluent collected, evaporated to dryness in a clean 10 ml beaker, and examined for a residue. This procedure is repeated if a residue is observed.

The benzene extracts and residues (if any) are combined and reduced to a few milliliters on the steam bath under a stream of dry air or nitrogen. The solution is transferred with the aid of 4 or 5 benzene rinsings to a coarse sintered glass funnel filtered into a tared aluminum pan (5 cm diameter, 1.2 cm deep) and the solvent evaporated on the steam bath under a gentle gas stream until the pan is constant in weight.

Results and Discussion

The unsaponifiable matter from 4 lots of butteroil was recovered by the procedure described. A minimum of 4 replicate analyses of each oil was performed. The samples of oil studied were obtained in identical manner from mixed herd milk (Beltsville, Maryland) and had been stored at −15C for various periods of time. Table I gives the results of this study and shows that good agreement between the replicates of each sample was obtained. The unsaponifiable matter of butteroil is given by Jenness and Patton (3) to be in the range of 0.30 and 0.45% of the weight of the fat.

In preliminary work, the completeness of saponification was verified by the hydroxamic acid test (4) on the isolated unsaponifiable matter. Within the limits of sensitivity of this test, the presence of any triglycerides was not indicated. As an additional

TABLE I

Percent Unsaponifiable Matter Found in Various Samples of Butteroil

Trial No.	Butteroil [a]			
	A	B	C	D
	% NS[b]	% NS[b]	% NS[b]	% NS[b]
1	0.34	0.34	0.33	0.33
2	0.36	0.33	0.33	0.35
3	0.35	0.34	0.34	0.34
4	0.36	0.34	0.33	0.33
5	0.34		0.34	0.33
6				0.34

[a] A-prepared on 7/9/63, B-prepared on 10/16/63, C-prepared on 11/18/64, D-prepared on 7/14/65.
[b] NS = unsaponifiable matter.

check, 4–5 g of tricaprylin was saponified as described and less than 0.5 mg of residue was obtained. The latter also gave a negative hydroxamic acid test. Furthermore, each milligram of triglyceride surviving saponification of 5 g of fat would raise the value of the unsaponifiable matter by 0.02%. The excellent agreement between replicate, of a given sample suggests, on this basis, that saponification was complete.

Cholesterol is the main constituent of the unsaponifiable matter of butteroil (3), comprising well over 90% of the weight of that fraction. Recovery studies of cholesterol added to butteroil were, therefore, undertaken. The sterol was recrystallized several times from hexane and alcohol and dried thoroughly. The purified sterol was added at levels of 8.1, 15.4 and 37.7 mg to the oil in the mortar, the remainder of the procedure being identical. Recovery of the sterol based on differences in the weight of the unsaponifiable matter of butteroil samples and the same oils with added cholesterol was 96, 106, and 103%, respectively.

The feasibility of scaling up the procedure was also investigated. For this purpose all of the reagents were increased proportionally and the size of the sintered glass funnels and mortars was chosen so that all operations could be conveniently conducted. Results shown graphically in Figure 1, indicate that good recoveries can be expected in the range studied.

No attempt was made to scale the procedure up further since the equipment (i.e., the funnel and mortar) would have to be quite large to handle weights of fat over 30 or 40 g. Assay of samples of oil smaller than 4 g was not tried.

Although our sole interest in pursuing the development of the method was for the analysis of butteroil, the method could, in all probability, be used for obtaining the unsaponifiables from other lipids either per se or by using appropriate modifications. In this connection, it should be mentioned that the saponification time and temperature (20 min at 100C) and the use of anhydrous $CaCl_2$ were chosen arbitrarily from the outset.

It is felt that the procedure described above may have a number of advantages over the Official American and British procedures. Besides the advantage which motivated development of the method (i.e., determination of hydroxyl components), it is faster, more convenient as far as equipment is concerned, and probably most important of all, the troublesome emulsions usually encountered in the classical methods are completely avoided.

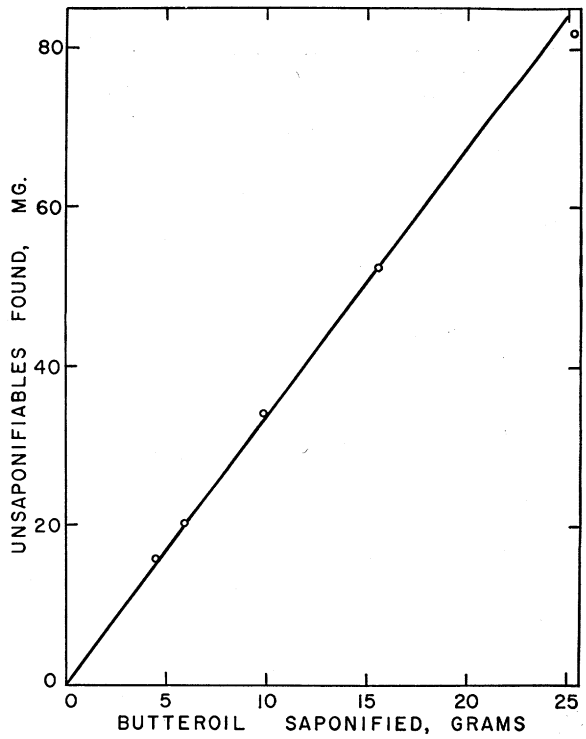

FIG. 1. Relationship between amount of fat saponified and unsaponifiable matter found.

The main disadvantages of the method are the limitations in the size of the sample which can be analyzed and the difficulty which might be met if it were desired to run the saponification in an inert atmosphere.

REFERENCES

1. Mehlenbacher, V. C., "The Analysis of Fats and Oils," Garrard Press, Champaign, Ill., 1960.
2. Nesheim, S. Private communication, Food and Drug Administration, U.S. Department of Health, Education and Welfare, 1964.
3. Jenness, R., and S. Patton, "Principles of Dairy Chemistry," John Wiley and Sons, Inc., New York, 1959, p. 41.
4. Shriner, R. L., R. C. Fuson and D. Y. Curtin, "The Systematic Identification of Organic Compounds," John Wiley and Sons, Inc. New York, 1964, p. 135.